REMARKS

Claims 1-17 were pending when the present Office Action was mailed February 27, 2007. In this Office Action, claims 1-17 were rejected and no claims have been allowed. More specifically, the status of the application in light of the Office Action is as follows:

(A) Claims 1-17 are rejected under 35 U.S.C. 103(a) over the combination of Cejpek et al., Simplified Extraction and Cleanup Procedure for the Determination of PAHs in Fatty and Protein-Rich Matrices, (1995) ("Cejpek"); Williams and Macrae, Non-Aqueous Size –Exclusion Chromatography Coupled On-Line to Reversed-Phase High-Performance Liquid Chromatography, (1989) ("Williams"); and Krishen and Tucker, Gel Permeation Chromatography of Low Molecular Weight Materials with High Efficiency Columns, (1977) ("Krishen").

A. Response to 103(a) Rejections

Claims 1-17 were rejected under 35 U.S.C. § 103(a) over the combination of Cejpek, Williams and Krishen. The Office Action concedes that Cejpek differs from the claims by not teaching interfacing of the gel permeation chromatography with the HPLC determination apparatus. The Office Action further acknowledges that Cejpek does not teach the use of tetrahydrofuran as the solvent in the gel permeation chromatography process. The Examiner asserts, however, that "it would have been obvious to one of ordinary skill in the art at the time the invention was made to perform the method of Cejpek in an automated manner as taught by Williams because of the close relationship between size exclusion chromatography and gel permeation chromatography as taught by Williams." Furthermore, the Examiner asserts that use of the column packing material and tetrahydrofuran as the elution solvent in the gel permeation separation step of Cejpek, as taught by Williams and Krishen, would have been obvious to one of ordinary skill in the art at the time the invention was made. For the reasons explained below, however, the applicants respectfully disagree and stress that Williams and Krishen fail to cure the deficiencies of Cejpek in order to support a Section 103 rejection of claims 1-17.

It would not have been obvious to automate the process for polycyclic aromatic hydrocarbon (PAH) determination in fatty and protein-rich food samples as taught by Cejpek. The Examiner cannot merely pick and choose various elements from the prior art to come up with the claimed combination of elements without regard for the functionality of the resulting combination of elements. As discussed below, the resulting combination of elements would render the process of Cejpek unsatisfactory and/or useless. Furthermore, the combination of Cejpek and Williams teaches away from the features of the claimed invention.

1. Independent Claim 1 is Directed to a Method for Determining the Level of One or More PAH in a Sample Selected from Edible Oils, Edible Fats, and Related Matrices

Independent claim 1 is directed to a method for determining the level of at least one PAH in a sample selected from edible oils, edible fats, and components thereof. The method includes providing a sample in a first solvent in which each PAH is soluble. The method further includes applying the sample to a gel permeation chromatography (GPC) column and eluting the sample with a GPC eluting solvent, effective to provide a fraction containing the PAH and which is substantially free of triglyceride and free fatty acid components of the sample. The method also includes injecting the fraction, without isolation, into a GPC/HPLC interface, wherein a solvent in which each PAH has low solubility is added to the fraction. The method further includes transferring the fraction, without isolation, onto a reverse-phase high performance liquid chromatography (HPLC) column, and initially eluting the fraction with a solvent in which each PAH has low solubility. The method also includes separately eluting each PAH to be detected from the HPLC column with an HPLC eluting solvent, detecting at least one PAH, and determining the level of the at least one PAH in the sample.

2. The Cited References

Cejpek discloses an extraction and cleanup procedure for determination of PAHs in fatty and protein-rich matrices. The procedure includes homogenization and ultra-sonication of solid food and fats in chloroform (approximately 150 ml total) to extract both PAHs and lipids. Following extraction, Cejpek teaches evaporation of chloroform from the sample on a heated rotary vacuum evaporator to about 3 ml total volume. (Cejpek, pg. 68-69, *Procedure C*). Following evaporation, Cejpek teaches cleanup of the sample on a GPC column, wherein the sample is loaded on the column and eluted with chloroform. After discarding a first 16.5 ml, a second 8.5 ml fraction (e.g., in chloroform) having the PAHs were collected and evaporated nearly to dryness. (Cejpek, pg. 69, *Cleanup*). After the chloroform was left exposed to evaporate spontaneously, the dry residue was resuspended in 0.2 ml acetonitrile. The resuspended fraction was analyzed by HPLC with fluorescence detection. (Cejpek, pg. 75-76, *Identification and quantitation*). Cejpek discloses use of acetonitrile/methanol/water mixtures in the HPLC determination process.

Williams discloses a method for non-aqueous size-exclusion chromatography (SEC) coupled on-line to reversed-phase HPLC (RP-HPLC) for determination of contaminants in food. Samples were dissolved in toluene, separated by SEC, and eluted in tetrahydrofuran. (Williams, pg. 318, *Size exclusion*). Williams teaches an interface between the SEC step and the RP-HPLC analysis, which includes eluting and isolating the required SEC fraction in tetrahydrofuran followed by continuous dilution of the sample in water prior to RP-HPLC gradient elution. (Williams, pg. 318-319, *Interface conditions – Reversed phase*). Williams discloses that the interface process can be automated.

Krishen teaches a gel permeation chromatography method using small-pore packing materials for increased resolution of low molecular weight analytes, including aromatic and cyclic hydrocarbons (Krishen, pg. 898, abstract; pg. 900, paragraph 3). Krishen reports using tetrahydrofuran to dissolve and elute samples. (Krishen, pg. 899, *Procedure* and *Figure 2 legend*).

3. The Combination of Cejpek, Williams and Krishen Cannot Support a Section 103 Rejection Because the Resulting Process Would be Unsatisfactory and/or Useless

It is improper to combine Williams' automated interface used to transition fractions between SEC cleanup steps and contaminant determination on HPLC with the PAH cleanup and determination procedure of Cejpek because the resulting process would render Cejpek unsatisfactory or useless for its intended purpose. For example, the primary inventive aspect disclosed in Cejpek is the use of chloroform for a direct extraction of PAHs from meat products and other fatty products, followed by a simple cleanup and fractionation of PAHs and lipids with GPC using chloroform (in which PAHs have high solubility) as the mobile phase. However, one of ordinary skill in the art would recognize at the time of the invention that GPC solvents or mobile phases (e.g., chloroform) are not good HPLC solvents because they are strong eluents on the HPLC column. (e.g., Williams, pg. 317, paragraph 2). To automate this transition, Williams teaches the necessity of continuous dilution of the fraction in water before being able to introduce the fraction to a HPLC column. (Williams, pg. 319, paragraph 3). However, chloroform is immiscible in water; therefore, based on the applicant's understanding, the dilution step of Williams would make Cejpek's procedure inoperable for detection and analysis of PAHs following HPLC. Accordingly, Cejpek's procedure includes the non-automated evaporation step between GPC and HPLC process steps and provides no motivation or suggestion to modify its teachings in view of Williams or in view of the knowledge generally available to one of ordinary skill in the art to arrive at the combination of elements in claim 1. If Cejpek was combined with Williams, it appears that PAH losses would be significant due to non-mixing of the chloroform and water solvents, and premature elution of the analytes due to the presence of chloroform. The rejection of claim 1 over the proposed combination of Cejpek and Williams should accordingly be withdrawn.

Claim 1 is further patentable under Section 103 because one of ordinary skill in the art would not combine the interface technique requiring sample dilution in water as taught

by Williams with a process directed to achieving the highly desirable detection at sub-ppb levels of PAHs. Indeed, Williams concedes that "the extent of dilution with water required for the SEC peak to be retained on the reversed-phase column is an important system parameter...and the volume of water required for purging could be a constraint upon achievable sensitivity depending upon its purity." (Williams, pg 322, paragraph 1). As a result of the water dilution step, Williams reports, on page 324, detection limits of about 0.5 mg/kg (about 50 ppb) which are significantly higher than the process of Cejpek and, more importantly, greater than FEDIOL approved detection limits (see Application, pg 2, lines 4-6). Therefore, one of ordinary skill in the art would not have been motivated to combine the process of Cejpek with the interface technology of Williams for determination and analysis of PAHs.

Claim 1 is patentable over Cejpek in view of Williams because the processes of Cejpek and Williams do not suggest that elimination of the evaporation step would yield lower detection limits of PAHs to sub-ppb levels or even to FEDIOL (Féderation de l'Industrie d'Huilerie de la Communauté Européenne) approved detection limits. Additionally, Cejpek had purported to develop a "new rapid method for the PAH determination" but had not considered the possibility of removing the evaporation step. (Cejpek, pg. 66, paragraph 2). Accordingly, the combination of Cejpek and Williams teaches away from the features of the claimed invention (e.g., injecting the fraction having at least one PAH, without isolation, into a GPC/HPLC interface, and transferring the fraction, without isolation, onto a reverse-phase-HPLC column). Specifically, a person of ordinary skill in the art at the time the invention was made would not recognize that the automatic analytical in-line method of the present invention would yield desirable detection levels for 20 different PAHs, and therefore, the results are surprising. Krishen fails to cure the deficiencies of Cejpek because it does not teach elimination of the evaporation step, nor does it teach in-line coupling of GPC and HPLC process steps. Accordingly, the Section 103 rejection of claim 1 should be withdrawn.

Claims 2-17 depend from claim 1. Therefore, the Section 103 rejection of these dependent claims should be withdrawn for the reasons discussed above and for the additional features of these claims.

B. Conclusion

In view of the foregoing, the pending claims comply with 35 U.S.C. § 112 and are patentable over the applied reference. The applicants request reconsideration of the application and respectfully submit that all of the claims are in condition for allowance. If the Examiner has any questions or believes a telephone conference would expedite prosecution of this application, the Examiner is encouraged to call the undersigned representative at (206) 359-8118.

Payment of the 3-month extension of time petition fee is submitted via EFT Account No. SEA1PIRM. If any additional fee is due, please charge our Deposit Account No. 50-0665, under Order No. 334498004US from which the undersigned is authorized to draw.

Dated: August 27, 2007

Respectfully submitted,

By____________________

Kellie S. Bickel
Registration No.: 46,386
PERKINS COIE LLP
P.O. Box 1247
Seattle, Washington 98111-1247
(206) 359-8000
(206) 359-7198 (Fax)
Attorney for Applicant